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Targeted Mutation Panel in NSCLC: A Single Center Experience

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Abstract

Introduction: Non Small Cell Lung Carcinoma (NSCLC) is one of the top ranking cancers in both males and female patients across India. Majority of cases are in advanced stage at presentation.

Recent advances in molecular techniques and drug discovery have made the mutation analysis of this carcinoma a part of standard investigation before treatment in NSCLC. In view of cost constraints, a targeted gene panel test is advised for all cases.The purpose of this study is to document the application of this practice at a single tertiary cancer care center in central India.

Material and Methods: Two hundred and thirty one cases of NSCLC were reported at our hospital from January 2019 till December 2023. Triage testing was done initially for Epidermal growth factor receptor (*EGFR*) gene mutations by ARMS RT-PCR/ Targeted NGS. Subsequently *EGFR* negative cases were tested for Analysis of Anaplastic lymphoma receptor tyrosine kinase gene (*ALK)*, Repressor of Silencing 1 (*ROS1*) gene by immunohistochemistry (IHC).

Results: 106 cases were found to have tested positive for these mutations, 56 were males and 50 were females. The average age for male patients was 60.17 yrs and for female patients was 56.3 yrs.

EGFR mutations were found in 40.69% cases, out of which exon 19 deletion and exon 21 L858R were the most frequent (38.09%).

Rare cases of single and compound mutations were also seen.

ALK and *ROS1* gene rearrangements were found in 4.76% and 0.43% respectively.

EGFR, ALK and *ROS1* mutations were mutually exclusive.

Conclusion: Targeted gene mutation testing is a very significant investigation in patients with advanced NSCLC, as it results in tailoring and optimising the treatment for individual patients and offers hope for the best outcome.

Keywords: Mutation test, Lung carcinoma, *EGFR, ALK, ROS1.*

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INTRODUCTION

Lung Cancer is one of the top ranking cancers in Indian population.[1]

Non Squamous Cell Lung Carcinoma (NSCLC) is the most frequent type of Lung Carcinoma found in India.[2,3] The majority of patients usually present in advanced clinical stage, with metastatic disease. Amongst the NSCLC, adenocarcinoma is the most frequent subtype, followed by Squamous cell carcinoma, Large cell Carcinoma and Adenosquamous carcinoma.

Since the availability and proven superiority of targeted therapy over standard chemotherapy, molecular testing has become the standard of care for patients of advanced NSCLC. As the cost of testing for all mutations by WES /WGS is still significantly high, clinical practice relies on testing for a targeted panel of the most frequently found somatic mutations by RT PCR/ IHC technique.

Many studies from India have found that *EGFR, ALK* and *ROS1* are the most frequently found mutations in Indian patients.[4-8]

EGFR-Epidermal Growth Factor Receptor (*EGFR*) is a cellular transmembrane receptor protein. The activation of *EGFR* plays an important role in cellular tumor growth proliferation and metastasis spread. *EGFR* tyrosine kinase (TK) gene mutations have been identified in non-small cell lung cancer (NSCLC) cases.[9]

Patients who test positive for these mutations, when treated with appropriate oral tyrosine kinase inhibitors (TKIs-eg: Gefitinib or Erlotinib) show improved and durable outcomes alongwith lesser toxicity and better quality of life as compared to patients on conventional chemotherapy.[10-12]

ALK: Genomic rearrangements in *ALK* can drive aberrant proliferation and tumorigenesis in several cancers including non-small cell lung cancer (NSCLC). Literature suggests, 3-7% of *ALK* fusions are observed in lung cancers.[13] As per the NCCN guidelines, presence of this fusionconfers sensitivity to first and second generation *ALK* tyrosine kinase inhibitors (ALK TKIs): Crizotinib, Ceritinib, Alectinib, Brigatinib.[14]

ROS1: *ROS1* gene rearrangements are reported in 1-2% lung adenocarcinomas and are associated with response to multitargeted TKI Crizotinib.

Both *ALK* and *ROS1* rearrangements are detected by FISH, however immunohistochemistry (IHC) is an alternate screening modality with high sensitivity and specificity.[15] IHC results are reported as H Score. Because gene rearrangements are rare, sensitive IHC screening is reasonable before ISH.

This study reports the occurrence of *EGFR, ALK* and *ROS1* mutations in patients of NSCLC from central India for the first time.

MATERIAL AND METHODS

Patient selection and Testing process: This is a retrospective observational study in patients tested for five years, from January 2019 to December 2023.

The tumor tissue samples of 231 advanced NSCLC patients were tested for mutations in *EGFR, ALK* and *ROS1* genes.

The 221/231 samples were in the form of Formalin fixed paraffin embedded (FFPE) tumor tissue blocks and the other 10 were peripheral blood collected in Streck tubes (liquid biopsy).

Considering the cost of genetic testing, triaging was done by performing the *EGFR* mutation test first, if it came positive, no further testing was done. If the *EGFR* mutation test was negative, *ALK* and *ROS1* mutation testing was performed.

EGFR mutation test by PCR: DNA extracted from the tissue block sample was tested for the presence of hotspot mutations (Exon 19 deletion/ insertion, Exon 20 insertions and substitution mutations- G719X, S768I, T790M, C797S and Exon 21 mutations L858R and L861Q in the EGFR gene) using ARMS real-time PCR. The target exons were amplified with mutation specific primers. Mutations were reported according to HGVS guidelines for mutation nomenclature (www.hgvs.org) and according to the reference sequence NM_005228.[3]

Liquid Biopsy Next Generation Sequencing: In case the tumor tissue failed the qualitative criteria for PCR test, peripheral blood sample in Streck tube was collected and ciculation free DNA (cf DNA) was extracted. Lung cancer liquid biopsy panel was used to detect genomic alterations (SNVs, InDels, & Fusions) in 24 lung cancer related actionable genes including *EGFR, ALK* and *ROS-1.* cfDNA isolated from blood plasma was used to perform UMI-based target enrichment and sequencing using a custom capture kit. The QC passed libraries were sequenced to a minimum depth >20000X (pre-UMI) on validated Illumina sequencing platform and compressed to >2000X (post-UMI) for variant analysis. The sequences obtained were aligned to human reference genome (GRCh38/hg38)

using BWA program.[16,17] Somatic mutations were identified using UMI corrected Sention pipeline.[18] Only non-synonymous and splice site variants found in the coding regions of the genes* of interest were used for clinical interpretation. The mutations were annotated using in-house annotation pipeline (VariMAT).

The sequenced reads were pre-processed and aligned to the reference human genome (Genome Version: hg38). Gene fusions were detected by multiple programs with and without UMI-aware algorithms. The cutoff and parameters of the algorithms and pipelines were finetuned based on our validation samples. Reportable alterations were prioritized, classified, and reported based on AMP-ASCO-CAP guidelines.[19]

Immunohistochemistry (IHC): If the *EGFR* mutations were not found in the sample through PCR, further testing for *ALK* and *ROS1* fusion was done by IHC. The IHC was performed using Roche-Ventana Benchmark GX slide autostainer with primary antibody clone D5F3 for *ALK* and CellSignal D4D6 rabbit mAB for *ROS1.* the secondary reagents used were Optiview DAB with amplification.

RESULTS

A total of 231 samples from patients of non small cell lung cancer (NSCLC) were tested for mutational analysis in the *EGFR, ALK* and *ROS1* genes. Of these 221 were Formal in fixed paraffin embedded tumor tissue blocks and 10 were peripheral blood samples (cf DNA). The cohort included 145 male patients and 86 female patients with ages ranging from 28-86 years. The mean age was 60.17 years for males and 56.30 years for females.

The most common NSCLC subtypes in both sexes were adenocarcinoma, followed by squamous cell carcinoma, large cell carcinoma and adenosquamous carcinoma. (*Table 1*)

Table 1: Subtypes of NSCLC in present study

NSCLC	M	F	Total
Adenocarcinoma	135	81	216
Squamous cell carcinoma	8	4	12
Large cell carcinoma	2	0	2
Adenosquamous carcinoma	1	0	1
Total	146	85	231

Mutational Analysis, revealed that actionable mutations were present in 45.88% of all cases. Specifically, *EGFR* mutations were found in 40.69% of cases, *ALK* rearrangements in 4.76% and *ROS1* rearrangement in 0.43%. (*Table 2*).

Table 2: Mutations found in NSCLC in present study

Mutations	M	F	Percentage	Total
EGFR	49	45	40.69	94
ALK	7	4	4.76	11
ROS-1	0	1	0.43	1
Total	56	50	45.88	106

Among the *EGFR* mutations, the most frequent were exon 19 deletions (58 cases), followed by exon 21 L858R mutations (30 cases). Less common mutations included exon 20 insertions (2 cases) and exon 18 mutations (2 cases). Additionally, one patient had coexistent exon 18 and 21 mutations and another had coexistent exon 19 deletion and 20 insertion mutations. (*Table 3*)

Table 3: Types of EGFR mutations found in present study

Type of EGFR mutation	M	F	Total
exon 19 deletion	33	25	58
exon 21 L858R	15	15	30
exon 20 Ins	1	1	2
exon 18	1	1	2
exon 18 and 21	0	1	1
exon 19 del and 20 Ins	1	0	1
Total	51	43	94

The majority of exon 19 deletions were observed in adenocarcinomas with 32 cases in males, and 24 in females alongwith one case each in squamous cell carcinoma.

Similarly, most exon 21 L858R mutations were in adenocarcinomas with 14 cases in males, and 14 cases in females and one case each in squamous and adenosquamous carcinoma. Single and compound uncommon mutations were also predominantly seen in adenocarcinoma.

DISCUSSION

This study describes the mutational profile of 231 patients of NSCLC from a single tertiary cancer care center in Central India.

In the present study, mutations was detected in 106 (45.88%) patients. Out of these, *EGFR* mutations were the most frequent (40.69%), followed by *ALK* (4.76%). *ROS1* mutation was found in only one case. These findings are comparable with the studyconducted by Deka *et al.*, Kishore *et al.* and Kesri *et al.*[4,6,7] (*Table 4*)

Table 4: Comparison of EGFR, ALK and ROS1 mutations in different studies

Name of Study	EGFR mutations	ALK mutations	ROS1 mutations
Present study	45.88%	4.76%	0.43%
Deka *et al.*	56.14%	33.33%	4.54%
Kishore *et al.*	37.23%	5.23%	3.85%
Kesri *et al.*	29.89%	4.6%	1.14%

A meta-analysis of 26 different studies by Raman *et al.*[5] states that there is a wide range of frequency of these three mutations across different regions of India. (*EGFR* 11.9-51.8%, *ALK* 4.1-21.4%, *ROS1* 3.5-4.1%).

The mutation frequency in the other Asian countries as reported by Shi *et al.* (50.2%) in China, 47.2% in Hong Kong, 52.3% in the Philippines, 62.1% in Taiwan, 53.8% in Thailand and 64.2% in Vietnam.[20]

The *EGFR* test by RT PCR is designed to detect mutations in exon 18,19,20, and 21 of the *EGFR* gene. In the present study, the frequency of single common mutations (exon 19 del/exon 21 L858R) was 93.61%. Most of the cases had exon 19 mutation, of which exon 19 deletion mutation was the commonest one found in 61.70% of patients. Second most common mutation was L858R mutation on exon 21 found in 31.91% of cases. This finding is comparable to Singh *et al.*[21] who found the frequency of common single mutations (exon 19 del/ exon 21 L858R) to be 79.5%.

Both these mutations are associated with responsiveness to *EGFR* tyrosine kinase inhibitors: Gefitinib, Erlotinib and Afatinib.[22,23] Dacomitinib and Osimertinib are also approved as a first line treatment in these cases, and have shown to reduce the progression risk by 54% as compared to standard Tyrosine kinase inhibitor therapy.[24]

A single uncommon mutation in the present study was Exon 20 insertion (2 cases). The frequency of exon 20 mutations in the various Indian studies varied from 2.8% to 25.39%. Exon 20 mutations confer resistance to the classical TKI therapy that is given in cases which harbor *EGFR* mutations; thus, the detection of exon 20 mutation is therapeutically significant.[25-27] Despite initial challenges in targeting *EGFR* exon 20 insertions, over the past 5–10 years a number of emergent therapies and clinical trials have been specifically developed for this unique molecular subgroup. Amivantanab has been granted FDA approval in 2021.[28]

Uncommon and compound *EGFR* mutations have been described by Indian authors[21] and also were found in the present study.

Exon 18 mutation was seen in 2 cases while one case each with compound mutations (exon 18 and 21) and (exon 19 deletion and 20 insertion) were found in the present study. The appropriate treatment for such patients has not been clarified.[29]

ALK gene rearrangements were the next frequently occurring mutations (4.76%) in the present study, and are comparable to Kishore *et al.* (5.23%)[6] and Kesri *et al.* (4.6%).[8] One study by Deka *et al.* showed a much higher frequency (33.33%).[4]

ROS1 gene rearrangements are quite rare in various studies across India,[4,6,8] and the present study also had only one case.

EGFR, *ALK* and *ROS1* mutations were seen to be mutually exclusive.

CONCLUSION

NSCLC cases across India show a wide range in the occurrence of actionable and druggable mutations.

The two most common mutations found in 88 patients of NSCLC in the present study were in the EGFR gene-exon 19 deletion and exon 21 L858R mutation. All these patients were eligible for treatment with the standard tyrosine kinase inhibitors (TKIs).

Two patients were found to have exon 20 insertion mutation in the EGFR gene and since this mutaion confers resistance to the usual tyrosine kinase inhibitors, they were not suited for targeted therapy.

Eleven patients were found to have mutations in the *ALK* gene, and were eligible to receive *ALK* TKIs.

Only a single patient had mutation in the *ROS1* gene which made the patient eligible for multitargeted TKIs.

Targeted gene Mutation testing is a very significant investigation in patients with advanced NSCLC, as it results in tailoring and optimising the treatment for individual patients and offers hope for the best outcome.

LIMITATION

All the patients in the present study are on clinical follow up, however the Progression free survival and Overall survival remains to be compared between the two groups of mutation positive and mutation negative cases. This comparison will reflect the difference between the clinical outcomes of these two groups.

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